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ARBUSCULAR MYCORRHIZAE FUNGI AND SUSTAINABILITY OF ARTISANAL GOLD MINING WASTE DISPOSAL SITE REVEGETATION

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ABSTRACT

Mining areas is associated with barren, highly altered soil structure, poor soil nutrients and high heavy metals concentration. Those condition, in some way may influence plant and its microbial symbion adaptation in order to establish vegetation. Arbuscular Mycorrhizae Fungi (AMF) form symbiosis to plant and provide both side beneficial. Because of its importance, AM F are addressed as important aspect in revegetation. In this paper we describe about AMF present in Artisanal Gold Mining (AGM) waste disposal site and furthermore we review about AMF adaptation in facing climate change. AMF spore and colonization were observed under microscope. Generally, 5 AMF species from genus Glomus and Acaulospora had been found. AMF Spore density varied from 8-162 spore/ 150 gram soil. Root colonizations were found in 8 plant species with percentage range from very low to high category. The highest concentration of mercury (135,4 ppm), Pb (51,4 ppm), and Cyanide (5,6 ppm) is followed by supreme AM diversity (4 species), spore density (162/150 gram soil), colonization percentage (high category), and plant diversity (6 species). The plant diversity was decreasing along with the low diversity and abundance of AMF. This results indicate that indigenous AMF can colonizes and promote plant diversity in tailing and potentially can be used to promote revegetation. Elevated C seems play role in enchancing AMF colonization but in soil with low available nitrogen, AMF are indicated to promote carbon loss. Furthermore, higher temperature may result in lower glomalin, protein produced by AMF which function in water-stability of soil aggregate.

Keywords: Arbuscular Mycorrhizae Fungi (AMP), Artisanal Gold Mining (AGM), Revegetation, Climate change

INTRODUCTION

. Artisanal Gold Mining (AGM) or Pertambangan Emas Tanpa Izin (PETI) in Indonesian, defined as informal, unregulated, and mostly illegal mining activities. In one side, AGM has positive impact in promoting economic income but in other side, the simple technologies applied and lack of rehabilitation planning result in environmental destruction (Prasetyo et at. 2010; Yunianto and Saleh 2011). AGM produces hazardous waste called tailing which contain high concentration of mercury since mercury is used for gold extraction (Juhaeti et a1. 2009). Tailing from AGM perhaps is the main source of mercury contamination for soil and water since Hidayati et a1. (2009) found 61.54-598.14 ppm of mercury in tailing sample from Pongkor AGM. The present of high concentration of mercury in environment is dangerous because mercury could potentially transform into highly toxic form like methyl mercury (Herman 2006) and enter food chain resulting danger to human health and welfare (Yu et a1. 2010).

Phytoremediation and revegetation in contaminated sites is one of the solution to limit the mobility and bioavability of heavy metals. This action become more important because the climate change scenario is predicted can aggravate the spreading of heavy metal pollutans (Al-Tabbaa et al.2007). As consequences of double stress caused by decreased soil properties and high concentration of heavy metal plus climate change pressure, revegetation and phytoremediation of contaminated soil must address the importance of microorganism in the process.

AMF is a group of fungi which has mutualistic symbiosis with 80-90% of plant species around the world. AMF colonize plant by forming Mycorrhizal structures consist of vesicula, arbuscula, and intraradical hypha in roots system. This fungi also produce spore in soil as long term survival structure and important in species dispersal (Smith and Read 2008). AMF play significant role in vegetation establishment by protecting the root system from the toxicity of heavy metal, increasing the root access to water and nutrient by extradical hypha of fungal (Galli et al. 1994). Nevertheless, low level of AM in contaminated soil caused by mining activities leads to delayed plant establishment (Siddiqui and Pichtel, 2008; Quoreshi 2008). Gaur and Adholeya (2004) suggested that AMF inoculation to plant being used to revegetate contaminated soil is good solution to achieve successful vegetation establishment and the best approach was using the indigenous AMF found in contaminated soil.

This paper aim to descirbe result about AMF status in revegetated AGM tailing located in Pongkor, West Java, Indonesia. Those result will be combined with paper reviewed related to AMF-plant system behaviour under climate change. It is hoped this paper can present recent understanding about AMF related to revegetation in contaminted soil and its adaptability with some climate change parameter such as elevated C 2. and drought.

MATERIAL AND METHODS

A. Location

Study was conducted in Pongkor AGM. This mining site is administratively located in Bantar Karet Village, Nanggung Sub-district, Bogor, West Java, Indonesia.

B. Sampling Method

Sampling of soil and root was conducted in spot 1, spot 2, and spot 3. The three sampling spot was naturally vegetated tailing. Soil was taken around the root zone about 500 gram each spot. Plant sample was taken for identification and its roots were immersed in 70% ethanol while soil samples were air-dried.

C. AMF Identification

Spore extraction was performed by wet sieving and decanting technique (Brundrett et a1. 1994). Approximately, 50 gram of soil was dispersed in 250 ml of water and left for 15-45 seconds then suspension

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was decanted through sieves with pore diameter of 500, 250, 90, and 53 qm. The residue of each sieve was collected in container, mixed in water then transfered into centrifuge tubes for centrifugation at 2000 rpm for 2 minutes. The supernatant was removed and the residue was added with 50% sucrose solution then centrifugated at 2000 rpm for 30 seconds. Supernatant was poured in 53 qm sieve then rinsed and washed into plastic petri dish. AM spores were examied under stereomycroscope, mounted in glass object with PVLG solution to make spore specimen. AM spore identification and counting was done under compound mycroscope.

D. Root Colonisation

Root samples were stained using method by Koske and Gemma (1989). Root was cleared with water and immersed in 2,5% KOH and boiled at 90° C for 3 minutes. Then, the roots were rinsed in water and immersed in HCl l% for 1 hour. Next, roots were immersed in tryphan blue solution and boiled at 90° C for 5 minutes. The stained roots were cut into l cm long, 30 pieces of them were randomly picked for colonisation examination under compound microscope. Calculation of colonisation percentage was using the formula from Giovanneti and Mosse (1980):

$$\textit{Colonised Root Percentage } (\%) = \frac{Total\ colonised\ root}{Total\ examined\ root} \times 100\ \% \quad (1)$$

Root colonisation percentage was divided into category of very low (<l0%); low (10-20%); medium (20-30%); high (>30%) (Prasetyo et al. 2010).

E. Soil Analysis

Soil samples were taken to soil chemical laboratory of Pusat Penelitian dan Pengembangan Teknologi Mineral dan Batubara (TEKMIRA) for mercury, cyanide, and lead (Pb) analysis. These three pollutants were chosen based on the previous research conducted by Hidayati et at. (2009). Mercury analysis was conducted by AAS-VGA, Pb analysis was conducted by AAS, and Cyanide analysis was conducted by spectrophotomertry method.

RESULTS

A. Status of Mercury, Cyanide, and Pb in Pongkor AGM Study Site

This study indicated that the chosen sampling site in Pongkor AGM positively contaminated with mercury, cyanide, and Pb with different degree (Table l).

Table 1. Mercury, Cyanide, and Pb Concentration in Vegetated AGM Tailing in Pongkor

Pollutants	Spot 1	Spot 2	Spot 3
Mercury (ppm)	135,4	94,395	87,54
Cyanide (ppm)	5,6	3,21	3,21
Pb (ppm)	51,4	46	50,2

B. Present of Arbuscular Mycorrhizae Fungi (AMF) in Pongkor AGM Study Site

The present of AM in soil is indicated by AMF spore in soil and colonisation in plant's root. Highest AM spore density and AMF diversity was found in spot 1, followed by spot 3 and spot 2 (Table 2). There were 5 AMF species found in vegetated AGM tailing in Pongkor: *Acaulospora scrobiculata* Trappe, *Acaulospora* cf. *delicata* Walker, Pteiffer & Bloss, *Glomus* cf. *geosporum* (Nicolson & Gerdemann) Walker, *Glomus* sp1., and *Glomus* sp2 (Figure 1).

Table 2. AM Spore Diversity and Abundance in Vegetated AGM Tailing in Pongkor

AMF Species	Spot 1	Spot 2	Spot 3
Acaulospora scrobiculata	+	+	-
Acaulospora cf. *delicata*	+	-	-
Glomus cf. *Geosporum*	-	+	+
Glomus sp1	+	-	-
Glomus sp2	+	-	+
Total spore	162	8	29

Note: (+): present; (-): absent; Total Spore Number was Counted in 150 grams soil for each spot

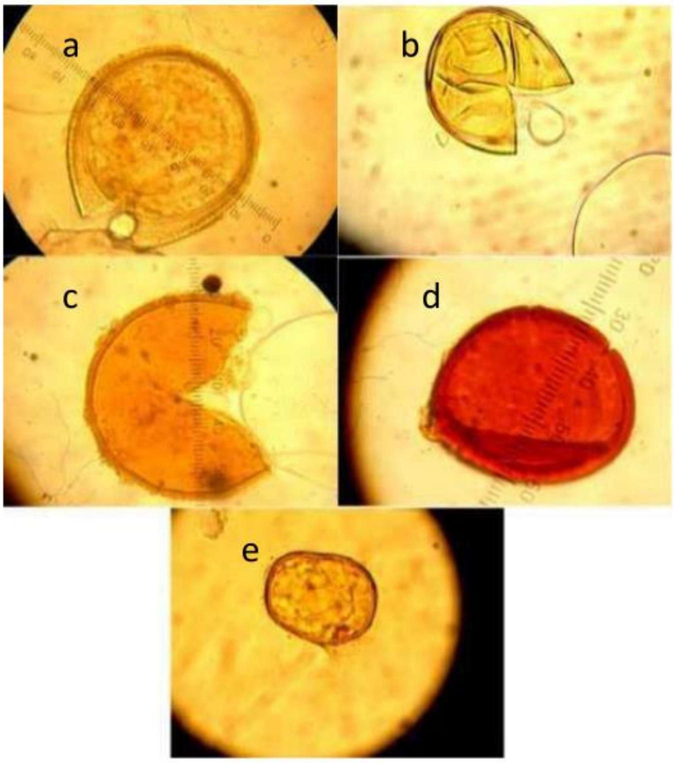

Figure 1. AMF Isolated from Vegetated AGM Tailing in Pongkor: (a) *Acaulospora .scrobiculata,* (b) *Acaulospora* cf. *delicate; (c) Glomus sp2.; (d) Glomus cf. Geosporum; (e) Glomus sp1.*

AMF Root colonization percentage and plant diversity were highest in spot l followed by spot 3 and spot 2. There were six plant species with high category of root colonization in spot 1 while the only one plant in spot 2, *Panicum repens* was in very low root colonization category. Three colonized plant species in spot 3 was included in very low-high category (Table 3).

Table 3. AM Root Colonization on Some Plant on Vegetated AGM Tailing in Pongkor

Plant Species	Spot	Colonization (%)	Category
Paspalum conjugatum	1	86,1	High
Eragrostis tenella	1	92,4	High
Eragrostis amabilis	1	58	High
Ageratum conyzoides	1	95	High
Sida rhombifolia	1	100	High
Mikania cordata	1	83	High
Panicum repens	2	3	Very low
Oryza sativa	3	10	low
Eleusine indica	3	16,5	Medium
Eragrostis tenella	3	56,67	High

Note: category of very low (<10%); low (10-20%); medium (20-30%); high (>30%) (Prasetyo et al. 2010).

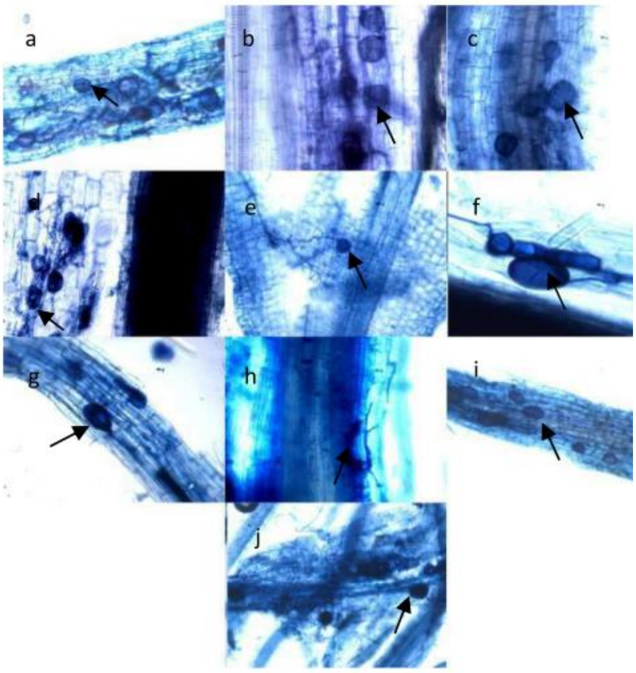

Figure 2. Root Colonisation of Plant Species Found in Revegetated Tailing in Pongkor AGM: (a) *Panicum repens;* (b) *Mikania cordata,* (c) *Sida rhombifolia,* (d) *Ageratum conyzoides;* (e) *Eragrostis amabilis;* (f) *Eleusine indica, (g) Eragrostis tenella* (spot 1); (h) *Eragrostis tenella* (spot 2), (i) *Paspalum conjugatum;* (j) *Oryza sativa;* Vesicula Organ Showed by Arrow

DISCUSSION.

Mercury and cyanide are two compounds used by AGM workers to extract gold from ore. Tailing, waste from gold extraction process usually being disposed directly to land and river. This processes contribute in increasing of pollutants mainly heavy metals ex. mercury and cyanide. Tomiyasu (2013) found mercury concentration around 13,5-55,6 ppm in Paddy field; 0,17-44,6 ppm in river sediment; and even 0,34- 27,8, ppm in forest soil (in Pongkor). This data indicate that mercury contamination sourced from AGM spread in large area in Pongkor. In normal soil, according to *British Columbia* (1995) standard, mercury concentration should be in range of 2- 10 ppm. Althought, there were some tailing dams near location but they are not sufficient for waste treatment since the lack space and maintenance. Furthermore, placing tailing in dams is just solution to degrade cyanide naturally but it still lefts amount of heavy metals in tailing.

Climate change is predicted to have direct or indirect effect on heavy metals mobility in soil and may hinder plant ability to adapt and make them more susceptible to stresses (Rajkumar et al. 2013). Furthermore, temperature increasing may lead to lowered soil organic matter, and increase soil erosion by wind and water (Brevik, 2013). In the case of contaminated soil, it also means of pollutants dispersal. The drought and heavy metal stress is likely to co-occur since tailing or polluted soil is associated with poor water holding capacity (Derome and Nieminen, 1998).

Arbuscular Mycorrhizae Fungi (AMF) are key component of vegetation establishment in contaminated area. Their occurances are well documented in several contaminated areas. In this research, total five AM F speceis are found in revegetated tailing diposal site. AMF genus *Acaulospora* and *GlomuS* found by Margarettha (2011) in ex-mining coal soil. *Acalauspora scrobiculata* and some species of G/omoS was found in AGM located in Sekotong, West Lombok (Prasetyo et a1. 2010). Early vegetation on industrial waste disposal site in nothern Poland had strong AMF colonized roots that indiicate this fungi is needed to help succession on the site (Turnau et al. 2006). All AMF originated from contaminated sites are considered having better tolerance than non-contaminated sites native strain. Shalaby (2003) suggested that AMF pollutants tolerant strain is likely come from phenotypic plasticity rather than genetic change of fungi. This means the contamination tolerance nature is temporal.

Althougth, sporulation is considered as the most sensitive parameter to long term metal stress (Biro et al. 2005), but AMF found in vegetated AGM tailing in Pongkor seemed not affected. Concentration of mercury, cyanide, and Pb did not effect the density of AMF spore number since the highest concentration of these three pollutans in spot 1 was followed by the highest AMF spore density. It was an inverse with study of Prasetyo et al. (2010) which suggest that spore density decrease along with the decreasing of soil properties and increasing of heavy metal concentration.

The other assesstment to detect AMF is by examination of root colonisation since this fungi is obligate symbion to plant (Bago et al. 2000). Root colonisation is the way AMF fungi interact directly to host plant in order to provide nutrient exhcange process via arbuscular structure. Althougth not all plants species form symbiosis with AMF, plant naturally grew in Pongkor AGM tailing is belonged to the family of plant which form symbiotic relationship with AMF (Poaceae, Asteraceae, and Malvaceae).

The highest root colonisation percentage was found in spot 1, followed by spot 3 and spot 2. Althougth it was located in same site, tailing in spot 1 was abandoned for a longer time than spot 2. The fresh tailing in spot 2 contribute to very low AMF root colonisation, spore density, and diversity both AMF and plant species. The other reason was probably as suggested Zubek et al. (2003) that in the same contaminated soil, better nutrients status and water holding capacity are distinguishing factor promoting the presence of AMF and plant establishment. Furthermore, relative AMF colonisaton and arbuscular formation can be used as indicator to show difference between restored and non-restored contaminated sites (Turnau et al. 2006). In this case, spot I was considered restored better than spot 2. Also, high diversity and abundance of AMF in spot 1 have impact in promoting plant biodiversity. This is consistent with Van der Heijden et a1. (I 998) suggestion that AMF diversity was major factor to maintain plant biodiversity in several ecosystem type.

Some weeds like *P. conjugatum, A. Conyzoides, M. cordata* found in study site was also found by Sambas et al. (2009) around AGM contaminated soil in Cikotok, Banten. Plants grow naturally like *P. conjugatum* can accumulate mercury and cyanide about 20 ppm in its biomass (Sambas et al. 2009). The establishment of early successional vegetation with high colonisation of AMF was indicated that the plant-AMF combination can promote naturally revegetation in AGM tailing.

The different colonisation percentage in one plant species is due to several factor such as compability of host plant and AMF species, and environmental factor. In fact, AMF colonisation to *A. conyzoides* (95%) in this study was even higher than the same species found in uncontaminated sites. *A. conyzoides* found in *Curcuma longa* orchard in Pakistan was only 6,5% (Tahira et al. 2012). High AMF colonisation in *A. zoides* plant in Pongkor AGM tailing is consistent to Turnau et a1. (2006)'s finding that root colonisation and arbuscular richness in *Festuca tenuifolia* collected from zinc waste was 2-5 times higher than in natural soil. It is possibly due to the plant dependency to AMF become higher in polluted soil.

Many study had identified the importance of indigenous AMF adapted to contaminated soil in promoting revegetation or phytoremediation. Leyval et a1. (1997) stated that AMF can decreased or increase heavy metal uptake by plants. *Berkheya coddii* plant colonised by AMF show increasing of biomass and Ni concentration in the drymass of the shoot (Turnau and Mesjasz-Przybylowicz 2003) meanwhile *Euphorbia milii* colonised by AM *Glomus fascicilatum* show increased Pb concentration in roots and inhibit translocation to shoot (Arisusanti and Purwani, 2013). In case of mercury phytoremediaton, Yu et a1. (2010) found that AMF increased of mercury in soil, decreased its accumulation in roots of maize. Thus, AMF provide optional result depend on the purpose of revegetation or phytoremediation whether to extract and dispose pollutants in safe place (phytoextraction) or stabilize and limititing pollutants mobilitation (phytostabilisation) (Cunningham et a1. 1995).

Althought present understanding related to AMF role in supporting plant growth (individual level) and vegetation establishment (community level)"are quite well studied but there are very few information about AMF adaptation to climate change. in fact, AMF adaptation to climate change still unconsistent among researches. Previously, AMF is considered could reduce atmospheric CO2 since its colonisation is increased under elevated atmpospheric COz (Hu et al. 1999; Gooday 1994). This is due to the plant demand of soil nutrients especially phosporus and water provided by AMF to balance the increased photosinthesis rate caused by high atmospheric C concentration (Rajkurmar et al. 2013). As the cost for nutrients and water absorption provided by AMF, plant allocates 10-20% of Carbon from its photosynthate for its fungi symbiont (Allen 1991) which means plant colonized by AMF will store more carbon in its rhizosphere than non-colonized plant.

Research conducted by Rillig e at. (2002) confirmed AMF hyphae length was increased about 40% followed by increasing colonization in warmed experiment plots. Review by Compant et al. (2010) suggest that elevated C 2 (indicator of climate change) usually enchance AMF colonization. Even though there is a trend of increaed colonization, it cannot be indicated that AMF can act as additional carbon storage like the previous hypothese. Cheng et al. (2012) suggested an opposite result that AMF under CO enhancement results in soil carbon losses, primarily in soil with low Nitrogen avaibility. The main driver of that phenomena is because AMF promote soil organic matter decomposition to gain available form of nitrogen (N H4") to fullfil plant's demand (Cheng et a1. 2012). Unfortunately, there is no related research which conducted in contaminated soil with similiar characteristic as AGM tailing. Since mercury (main issue of AGM soil contamination) can be immobilised and converted into non-available form by organic matter and humic acid in soil (Moreno et al. 2009), loss of organic matter might also means enchancement of mercury contamination dispersal into a wider area.

Another parameter affected by warming effect was glomalin, a protein produced by AMF that function in water- stability of soil aggregates (Rillig et al. 2002). Aggregate stability is important parameter in revegetation of contaminted sites since tailing is consist almost no organic matters with low water holding capacity (Zubek et al. 2003). Decrease in glomalin production may results in vulnerabilty of such area to erosion and pollutants dispersal. Also, low aggregate stablity helps accelerating soil carbon loss. Even so, AMF is still considered as potential tool that can enhance drought resistance of plants. This feature will be more important under climate change scenario since it is predicted that some areas of the world will be increased in dry periods (Compant et a1. 2010).

Research to understand AMF related to heavy metals and climate change is still solely conducted so far. There is no single publication that accomodate those three aspect; plants- AMF system, heavy metals contamination, and climate change paramaters together. Without such understanding, we have no idea about any mitigation action should be taken about AMF and revegetation management in contaminated land (i.e. AGM).

CONCLUSION

This research provide understanding about adaptation of AMF in AGM tailing disposal site which contain high amount of heavy metals mainly mercury. There are five AMF species found; *Acaulospora scrobiculata, Acaulospora* cf. *delicate, Glomus* cf. *Geosporum, Glomus* sp1, *Glomus* sp2 with AMF spore density is in range of 8-162 spore/ 150 grams soil. AMF colonization is found in 10 plant species with very low-high colonization cateogy. There is a trend that AMF can tolerate even the most polluted spot (1) and promote better plant diversity.

Reviewed paper showed that climate change will results mostly in higher AMF colonization. Despite the higher colonization, there are some unfavourable results in which AMF probably induce soil organic carbon loss. Warming effect also reduce glomalin production that function to stabilize soil aggregate. Futher impact of unstabilized aggregate may cause erosion and contamination (i.e heavy metals) dispersal. But still, AMF is considered as plant symbion to help in absorbing water in water scarcity condition caused by climate change.

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